DIURNAL CHANGES IN MITOTIC ACTIVITY AND GLYCOGEN CONTENT IN RAT LIVER DURING PROLONGED STARVATION

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After starvation for 7 days, the number of mitoses and the glycogen content in the liver of albino rats both diminish. However, the diurnal rhythm of mitotic activity and glycogen content persists.

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As earlier investigation showed that if animals are fed once daily at different times, the rhythm of cell division and the level of mitotic activity remain unchanged, whereas glycogen deposition depends on the time of taking food [6].

To continue the examination of possible relationships between mitotic activity and glycogen content in the liver, it was decided to investigate these relationships during changes in physiological activity of the organ, and especially during prolonged starvation.

Prolonged starvation inhibits mitotic activity [5, 7, 9]. However, the diurnal rhythm of mitotic activity during starvation has been inadequately studied. According to some reports, the diurnal mitotic rhythm persists during starvation [5, 7].

The object of the present investigation was to study the relationship between mitotic activity and glycogen content in the liver throughout the 24-h period under normal conditions and during prolonged starvation.

EXPERIMENTAL METHOD

Experiments were carried out on 67 male albino rats weighing 150 g. The group of starving animals (25 rats) was kept in individual iron cages without litter but with free access to water.

The control animals (42 rats) received natural food once daily. The food remains were removed next day, after which fresh food was given.

Seven days after the beginning of starvation, the experimental animals were sacrificed along with the controls at invervals of 4 h throughout the 24-h period (4-5 experimental animals, 7 controls at each time). Pieces of the left lobe of the liver were fixed in Carnoy's fluid. Sections were cut to a thickness of 7 μ and stained with hematoxylin-eosin. Mitoses were counted in 14,000 liver cell nuclei in the control series and in 25,000-30,000 nuclei in the experimental group. The mitotic index was expressed in pro mille. Glycogen was detected histochemically by Shabadash's method. Sections 5 μ in thickness were examined in the MF-4 microphotometer, the degree of absorption of light over the whole sections being measured. The results were read on the linear scale of the galvanometer, graduated up to 100.

EXPERIMENTAL RESULTS

At the time of sacrifice the mean weight of the control rats was 182.2 g; that of the fasting animals was 88.5 g. The loss of weight of the experimental animals compared with the controls was 51.4%. It was found at necropsy that the liver of the fasting rats was reduced in size and dark in color. Some of the animals died during the last few days of starvation.

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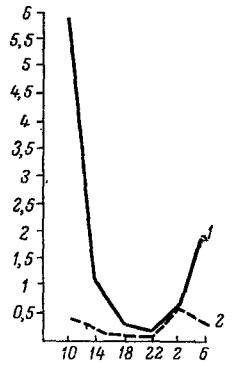

Fig. 1

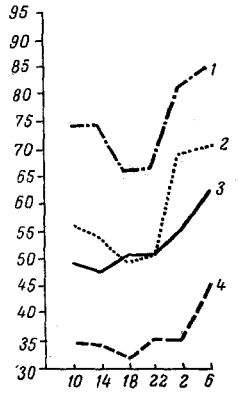

Fig. 2

Fig. 1. Changes in mitotic activity in parenchymal cells of rats' liver during 24-h period. Abscissa – time of day; ordinate – number of mitoses (in %); 1) control rats; 2) fasting rats.

Fig. 2. Glycogen content in parenchymal cells of rats' liver during the 24-h period. Abscissa – time of day; ordinate – conventional units; 1) control rats, peripheral part of section; 2) central part of section; 3) fasting rats, peripheral part of section; 4) central part of section.

Changes in mitotic activity in the parenchymal liver cells are illustrated in Fig. 1.

The curve of the diurnal rhythm of the mitoses in the liver of the control rats corresponds on the whole to the course of the curve obtained previously by other authors and by myself [2, 4, 6, 10]. The number of mitoses reached a maximum at 6 and 10 A. M. (1.66 and 1.09%) and a minimum at 6 and 10 P. M. (0.21 and 0.13%). The differences between the numbers of mitoses at 10 A. M. and 2 P. M. ($P = 0.01$), and also at 10 A. M. and 6 P. M. ($P = 0.004$) were statistically significant.

The mitotic activity in the experimental animals fell during fasting for 7 days. The mean diurnal mitotic activity for the control rats was 1.69% and for the fasting rats 0.23%, i.e., 7 times less. However, the number of mitoses in the fasting rats at 6 and 10 P. M. differed significantly ($P = 0.01$) from the number of mitoses at 2, 6, and 10 A. M. Hence, from the difference in the number of dividing liver cells at the times of the maximum and minimum of mitosis, it may be concluded that their diurnal rhythm persisted in the fasting animals.

The glycogen content in the liver of the control rats showed a well-marked diurnal rhythm (Fig. 2). It reached a maximum at 2, 6, and 10 A. M. In this period, the liver cells in the histological preparations were literally packed with glycogen granules. During the afternoon, the glycogen content began to fall, reaching a minimum in the evening, at 6 and 10 P. M. The differences between the glycogen content in the morning and evening were significant at all times ($P = 0.0001$).

During starvation for 7 days the liver glycogen content of the rats fell sharply below its normal level (at all times of fixation $P = 0.001$). The liver cells of the fasting animals were without glycogen granules in the evening, but at night and in the early morning (2 and 6 A. M.) tiny granules appeared and the preparations were stained pink. This accumulation of glycogen at night and in the early morning was statistically significant ($P = 0.008$). Consequently, during starvation for 7 days the diurnal rhythm of changes in the glycogen content persisted.

A significant decrease in the size of the nuclei and cells was observed in the fasting animals compared with those receiving a normal diet. At 10 A. M., the mean area of the cells in the control rats was 308.1 μ^2 and that of the nuclei 44.4 μ^2, while the corresponding figures for the fasting rats were 142.7 and 39.8 μ^2.

Hence, during starvation for 7 days, the diurnal rhythm of mitoses persists in the liver of albino rats. Persistence of the diurnal rhythm of mitoses in the epithelium of the gastric mucous membrane of these same rats was demonstrated previously [7].

Our results showing persistence of the diurnal rhythm of the liver glycogen content are in agreement with those obtained by workers [8] who investigated the liver of rats fasting for 2 days.

The experimental results described above show that under conditions of prolonged starvation the relationship between mitotic activity and glycogen content in the liver remains the same as under normal conditions: a maximum of the number of mitoses corresponds to a maximum of the glycogen content.

It is interesting to note that in animals sacrificed at 2, 6, and 10 P. M. and 2 and 6 A. M., the differences between the numbers of mitoses in the experimental and control rats were not significant. Consequently, if the investigation had been carried out only at these times, the erroneous conclusions would have been reached that during prolonged starvation the mitotic activity does not diminish. It is only by taking into account the diurnal rhythm of mitoses that the correct conclusion may be drawn regarding the character of the change in mitotic activity [3].

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